DEVELOPMENT OF *HYLA ANNECTANS* JERDON, 1870 FROM NAGALAND, INDIA[1]

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(*With four plates*)

Key words: *Hyla annectans*, ontogenic stages, normal development, Nagaland, laboratory condition, metamorphosis

Hyla annectans breeds during May to July in temporary rain pools, terraced fields and other waterlogged areas (depth 5-7 cm). Normal development has been studied from the egg through metamorphosis for four breeding seasons (1996-2000) under laboratory conditions. The time required for completion of a life cycle varies under different weather conditions in the field. In the laboratory (16-22 °C) the frog completed its life cycle in 64 days, 14 hours.

Introduction

Studies on the successive ontogenic stages, to record the normal developmental table, are important in understanding the ecology of a species and for planning conservation measures.

Various authors (Dutta and Mohanty-Hejmadi 1976, Mohanty-Hejmadi and Dutta 1977, Agarwal and Niazi 1977, Roy and Khare 1978, Mohanty-Hejmadi *et al.* 1979, 1980, Kiyasetuo and Khare 1986, Dutta *et al.* (1990-91) have contributed to the study of normal developmental tables of Anuran amphibians in India.

In Nagaland (25° 15'-27° 4' N, 93° 20'-95° 15' E), *Hyla annectans* is distributed along the Borail range at various altitudes 1,400-2,440 m above msl. Average atmospheric temperature during breeding season ranges from 16.5-26.6 °C. Relative humidity ranges from 74.33-81.63%. Precipitation ranges from 128.23-428.33 mm. Water temperature varies from 14-28.5 °C. Breeding activity starts from the middle of April and lasts till July end. The frogs breed in water logged places like temporary ponds, rainpools, puddles and terraced paddy fields. Embryonic development was observed for a period of four breeding seasons (1996-2000). Laboratory rearing was carried out in the Department of Zoology, Kohima Science College, Kohima, Nagaland.

Methodology

Amplexing pairs were collected from the field and transferred to aquaria or glass containers with water, allowing only half of the body to be submerged. Amplexing lasts for 3-5 hours. Eggs are laid between 0100-0400 hrs in the aquaria as well as in the field. Development stages were fixed in 5% formaldehyde solution; measurements were taken from preserved specimens. Photographs for Plates 1-3 were taken from preserved specimens, while for Plate 4 live individuals were used.

Observations

Breeding activity starts from the middle of April (12.3-26.6 °C) with the males' breeding call. Females appear only after one or two

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showers. In early May, amplexing pairs were collected and kept in glass containers or aquaria. Eggs were laid in batches in the field. In the aquarium, due to the absence of vegetation or other substratum, the jelly capsules of the eggs adhered together to form a single mass. The number of eggs laid by one female ranged from 590-650. Culture was maintained in clean enamelled trays and 100 fertilized eggs were stocked in each tray to avoid over-crowding. They were reared in the laboratory at 16-22 °C. Larvae were fed with *Spirogyra,* which is common in the breeding habitats. Tadpoles were staged according to Gosner (1960).

The no. of individuals per tray (Stocking number = 100) was reduced with progressive developmental stages.

Embryonic stage

Forty samples were measured to record the mean size of each stage (1-46).

Fertilization stages

Stage 1: *Fertilized egg* (Age 0 hrs; length 1.52 mm) (Plate 1, Fig. 1). The egg is spherical. The animal pole is pigmented dark brown, paling to white at the vegetal pole.

Stage 2: *One cell stage* (Age 0.55 hrs; length 1.52 mm). A lightly pigmented area, the grey crescent appears between the animal and vegetal pole towards the pigmented hemisphere.

Cleavage stages

Stage 3: *Two cell stage* (Age 1.50 hrs; length 1.52 mm) (Plate 1, Fig. 2). The meridional cleavage furrow originating at the animal pole proceeds to the vegetal pole and divides the egg completely into two equal blastomeres.

Stage 4: *Four cell stage* (Age 2.20 hrs; length 1.52 mm) (Plate 1, Fig. 3). The second meridional furrow, which starts at the animal pole, extends to the vegetal pole at a right angle to the first.

Stage 5: *Eight cell stage* (Age 2.45 hrs; length 1.52 mm). The third cleavage is latitudinal, slightly above the equator, which forms eight blastomeres. The four smaller micromeres of the animal pole are pigmented dark brown, whereas the four bigger macromeres of the vegetal pole are unpigmented.

Stage 6: *Sixteen cell stage* (Age 3.20 hrs; length 1.52 mm) (Plate 1, Fig. 4). The cleavage furrow is vertical. First, the pigmented micromeres are divided into eight cells, resulting in twelve cells (i.e. 8 micromeres and 4 macromeres). This is followed by the division of the four unpigmented macromeres as the cleavage furrow reaches the vegetal pole, resulting in 16 cells altogether.

Stage 7: *Thirty-two cell stage* (Age 3.52 hrs; length 1.56 mm) (Plate 1, Fig. 5). The latitudinal cleavage furrows of the micromeres and macromeres bring about the formation of 16 micromeres and 16 macromeres.

Stage 8: *Mid blastula* (Age 7-54 hrs; length 1.56 mm) (Plate 1, Fig. 6). The number of cells increased to more than 64 cells. The blastomeres are more numerous and smaller than before. The surface of the animal pole resembles a cluster of beads.

Stage 9: *Late blastula* (Age 12.10 hrs; length 1.56 mm). The surface of the animal pole has a granular appearance, which gradually becomes smooth. The pigmented region extends over the vegetal pole, which marks the beginning of the epibolic movement of the micromeres onto the macromeres.

Gastrulation stages

Stage 10: *Crescent dorsal lip* (Age 15.05 hrs; length 1.56 mm) (Plate 1, Fig. 7). The dorsal lip of blastopore has formed and is crescent-shaped. The unpigmented zone of the vegetal hemisphere is reduced due to continued migration of the pigmented micromeres towards the vegetal pole.

Ao, J. Meren *et al.*: *Hyla annectans* Jerdon PLATE 1

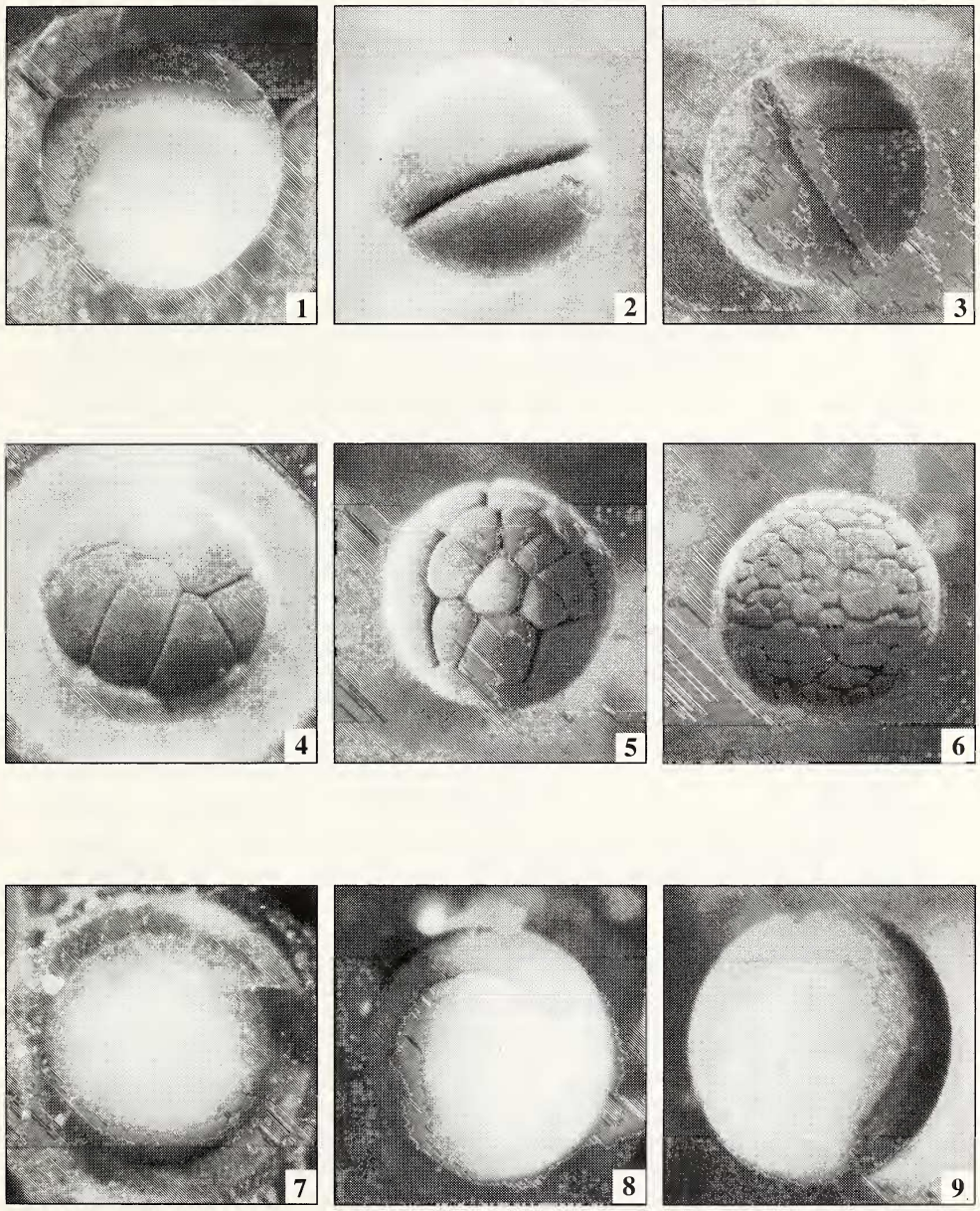

Fig.1 Fertilized egg, Fig. 2 Two cell stage, Fig. 3 Four cell stage, Fig. 4 Sixteen cell stage, Fig. 5 Thirty-two cell stage, Fig. 6 Mid blastula, Fig. 7 Crescent dorsal lip, Fig. 8 Horse-shoe shaped dorsal lip, Fig 9. Neural plate.

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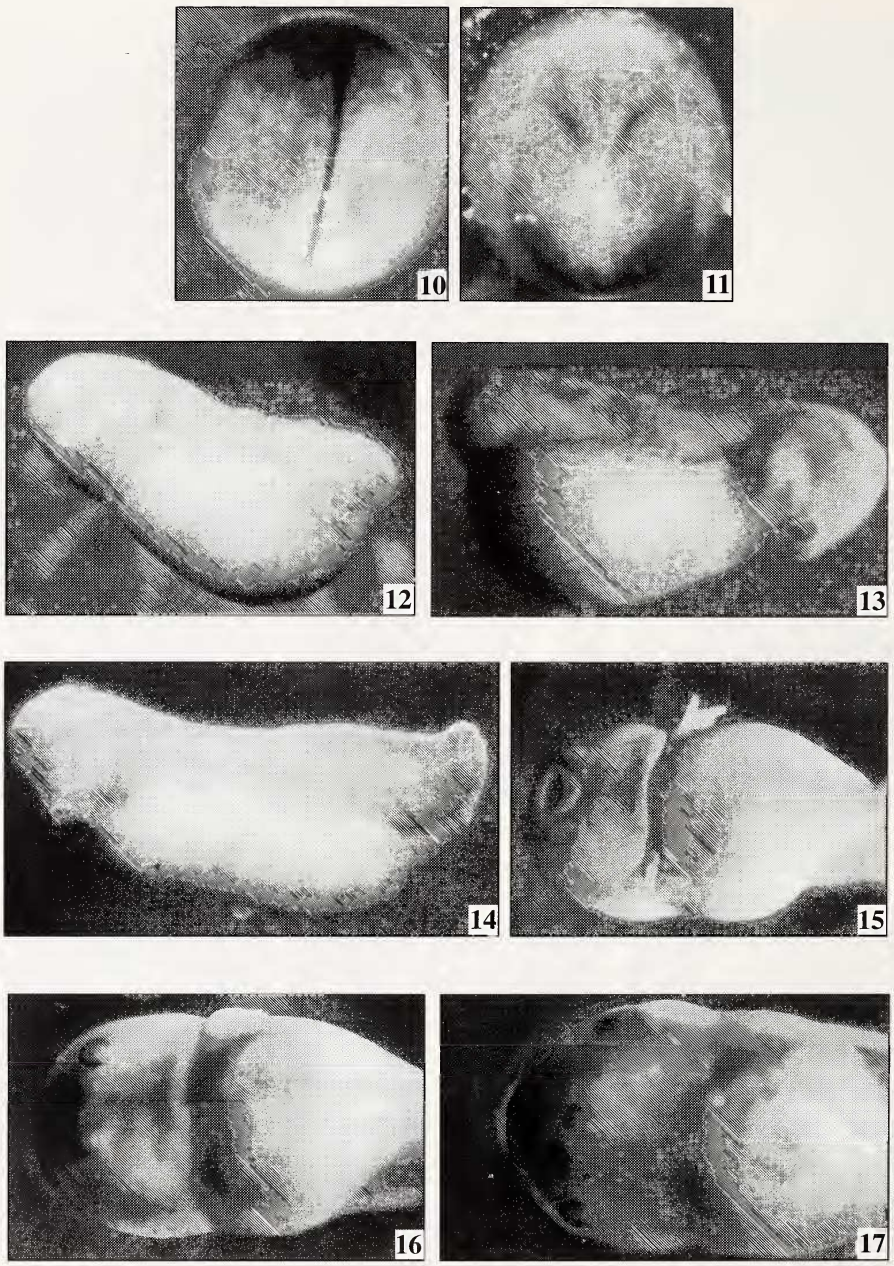

Fig. 10 Closure of neural fold, Fig. 11 Neural tube, Fig. 12, 13 Tail bud stage, Fig. 14 Muscular response stage, Fig. 15 Opercular stage, Fig. 16 Opercular fold of embryo closed on right side, Fig. 17 Operculum of embryo closed on left side

Stage 11: *Horse-shoe shaped dorsal lip* (Age 16.50 hrs; length 1.56 mm) (Plate 1, Fig. 8). The epibolic migration of micromeres over the vegetal pole reduces the exposed area of unpigmented macromeres, which is surrounded by the lateral lips of the semicircular or horse-shoe shaped blastopore.

Stage 12: *Small yolk plug* (Age 26.10 hrs; length 1.56 mm). The ventral lip of blastopore shifts to the posterior end. The uninvaginated macromeres, surrounded by the blastoporal lips, protrude a little and constitute the yolk plug.

Neurulation stages

Stage 13: *Neural plate* (Age 34.30 hrs; length 1.6 mm) (Plate 1, Fig. 9). The embryo is slightly elongated. The dorsal surface is flattened to form the neural plate, which is differentiated with the concentration of pigments along its borders.

Stage 14: *Neural fold* (Age 36.20 hrs; length 1.65 mm). The neural fold becomes distinct, with broad cerebral and narrow spinal cord regions of the neural plate. The neural folds gradually approach each other from blastopore to anterior region.

Stage 15: *Closure of neural fold* (Age 38.20 hrs; length 1.72 mm) (Plate 2, Fig. 10). The posterior end of the embryo becomes broader. The neural folds come closer and touch each other, both in the cerebral and spinal cord regions, forming a shallow neural groove, which is broader in the cerebral region.

Stage 16: *Neural tube* (Age 40.50 hrs; length 2.0 mm (Plate 2, Fig. 11) The neural folds have fused completely to form the neural tube, which is raised at the mid-dorsal ridge and demarcated by a darkly pigmented strand. The head and trunk are well marked. Gill plates appear as faint bulges. The increase in size of the embryo along with the associated vitelline capsule, is not accompanied by a similar increase in the length of the first envelope, and thus it ruptures at the anterior end.

Stage 17: *Tail bud stage* (Age 42.50 hrs; Trunk 2.44 mm; Tail 0.47 mm) (Plate 2, Figs. 12, 13). Tail bud appears at the posterior end of the embryo. It is wider than long, directed dorso-posteriorly and marked off from the body by a ventral notch. Stomodeal groove depression is slightly marked by a darker area. Bulges of the gill plates are distinct.

Stage 18: *Muscular response stage* (Age 60.00 hrs; Trunk 2.71 mm; Tail 0.75mm) (Plate 2, Fig. 14). Head region is well defined, with optic bulges and bulges of the gill plates. Oral suckers are indicated by two heavily pigmented elongated areas joined medially by a narrow lightly pigmented band below the stomodeum. Stomodeal depression is seen between the oral suckers. Due to gradual elongation of the embryo, the tail starts curving laterally to right or left, within the contour of the vitelline membrane.

Stage 19: *Heart beat stage* (Age 70.07 hrs; Trunk 3.10 mm; Tail 1.4 mm). Pulsation of heart is seen below and behind gill bud. Small pigmented depression at anterior end marks olfactory pit. Stomodeal depression becomes somewhat triangular. External gill buds prominent. Embryo coils to mechanical stimuli.

Stage 20: *Gill circulation stage* (Age 72.29 hrs; Trunk 3.28 mm; Tail 1.5 mm). Gills distinct, rudimentary branching at distal end. Oral suckers nipple-shaped. Stomodeal pit still a shallow triangular depression. Vitelline membrane becomes thin and weak. Anterior end of the head pressed against the vitelline membrane.

Post-embryonic development

Stage 21: *Larva hatched* (Age 102.29 hrs; Head and Trunk 3.2 mm; Tail 3.2 mm). The head causes a bulge in the vitelline membrane at the anterior end. At this point the membrane breaks and the larva emerges from the mass of jelly. Tail straight, tail fin dusky. Olfactory pit deepens, cornea begins to be transparent. Stomodeum now

a deep triangular pit, whose opening is the larval mouth.

Stage 22: *Tail fin circulation stage* (Age 117.29 hrs; Head and Trunk 3.4 mm; Tail 3.6 mm). Tail fin circulation starts at base of anterior part of dorsal fin, just above the trunk. Mouth slightly wider. Upper and lower labial fringes develop, but without papillation.

Stage 23: *Opercular fold stage* (Age 143.59 hrs; Head and Trunk 3.6 mm Tail 4.5 mm) (Plate 2, Fig. 15). Operculum covers bases of external gills. Jaws not keratinised. Upper and lower labial fringes develop papillae and faint labial ridges. Pigmentation on tail begins, cloaca not opened.

State 24: *Opercular fold of embryo closed on right side* (Age 172.29 hrs; Head and Trunk 3.7 mm; Tail 5.0 mm) (Plate 2, Fig. 16). Operculum closed on the right side. Labial jaws form supra- and infra-rostrodont. A faint row of upper supra-angular keratodont develops. Other characters as in Stage 23.

Stage 25: *Operculum of embryo closed on left side* (Age 185.29 hrs; Head and Trunk 4.2 mm; Tail 6.0 mm) (Plate 2, Fig. 17). Operculum closed and gills disappear; oral suckers diminishing. Spiracle formed. Tail lightly pigmented. Anal tube opens, tadpole starts feeding.

Stage 26: *Hind limb bud stage* (Age 356 hrs; Head and Trunk 7.0 mm; Tail 10.0 mm) (Plate 3, Fig. 18). Appearance of hind limb bud at a groove between the belly wall and base of tail on either side of cloacal tail piece. Length of limb bud less than half its diameter. Dental formula becomes 1:1 + 1/3. Pigmentation spreads to dorsal and anal fins.

Stage 27: *Length of limb bud equal to half its diameter* (Age 420 hrs; Head and Trunk 7.8 mm; Tail length 13.0 mm). Length of limb bud equal to half its diameter. The patches of pigmentation in the tail fin spread considerably.

Stage 28: *Length of limb bud equal to its diameter* (Age 514 hrs; Head and Trunk 8.4 mm; Tail 16.0 mm) (Plate 3, Fig. 19). Distal end of limb bud slightly conical.

Stage 29: *Length of limb bud is equal to one and half times its diameter* (Age 600 hrs; Head and Trunk 9.0 mm, Tail 18.0 mm). Distal end conical.

Stage 30: *Length of limb bud is equal to twice its diameter* (Age 684 hrs; Head and Trunk 11.0 mm, Tail 19.1 mm). Distal half of conical limb bud slightly bent ventrally. No pigmentation on limb bud.

Stage 31: *Foot paddle stage* (Age 748 hrs; Head and Trunk 11.5 mm; Tail 20.1 mm) (Plate 3, Fig. 20). The distal end of limb bud is flattened mediolaterally to form a foot paddle. Knee bend is prominent. Light pigmentation starts at outer base of limb bud.

Stage 32: *First indentation* (Age 813 hrs; Head and Trunk 12.0 mm; Tail 23.0 mm). The margin of the foot paddle becomes slightly indented on the dorsal side, which marks the prominences of the future 4th and 5th toes.

Stage 33: *Second indentation* (Age 853 hrs; Head and Trunk 12.8 mm; Tail 24.5 mm) (Plate 3, Fig. 21). The margin of the foot paddle becomes indented on the ventral side, behind the prominence of 4th toe, which marks the 3rd, 4th and 5th toes.

Stage 34: *Third indentation* (Age 890 hrs.; Head and Trunk 13.2 mm; Tail 26.0 mm) (Plate 3, Fig. 22). The margin of foot paddle becomes indented, on the ventral side, behind the prominence of 3rd toe, which marks the prominence of 2nd, 3rd, 4th and 5th toes.

Stage 35: *Fourth indentation* (Age 936 hrs; Head and Trunk 13.4 mm; Tail 26.4 mm.) (Plate 3, Fig. 23). The margin of the foot paddle is indented behind the 2nd toe demarcating the prominence of the 1st toe. All the five toes are separated from each other.

Stage 36: *Margin of 5th toe web directed towards the tip of 2nd toe* (Age 1019 hrs; Head and Trunk 13.6 mm; Tail 27.2 mm) (Plate 3,

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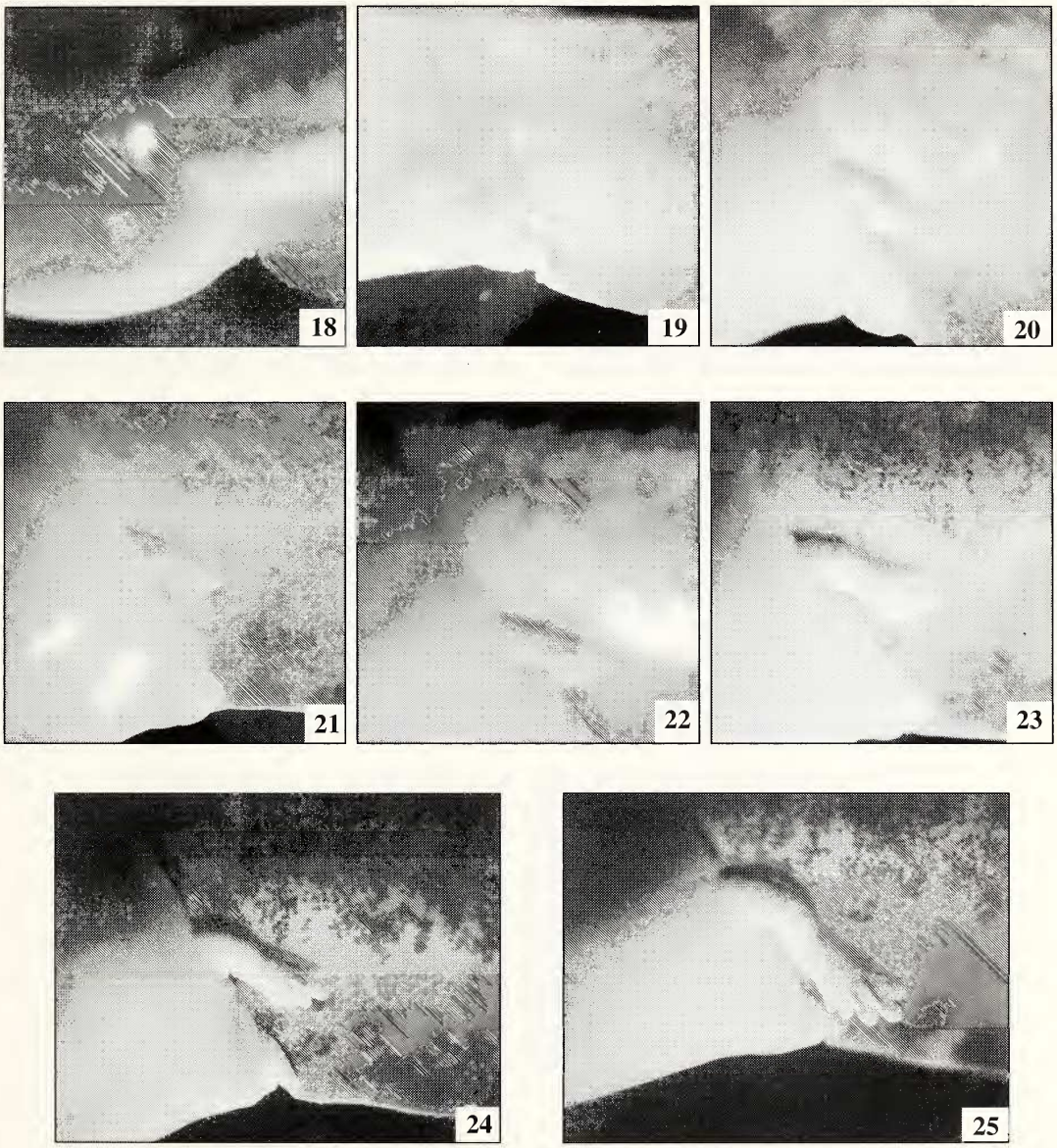

Fig. 18 Hind limb bud stage, Fig. 19 Length of limb bud equal to its diameter, Fig. 20 Foot paddle stage, Fig. 21 Second indentation, Fig. 22 Third indentation, Fig. 23 Fourth indentation, Fig. 24 Margin of 5th toe web directed towards the tip of 2nd toe, Fig. 25 Margin of 5th toe web directed towards prehallux.

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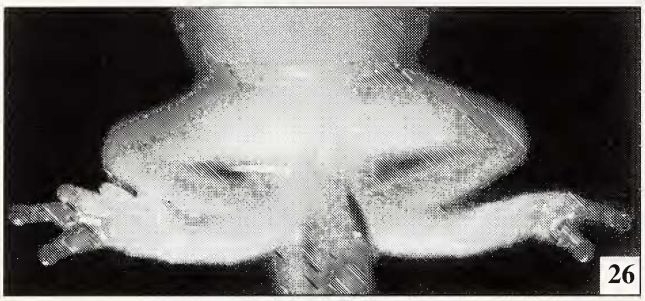

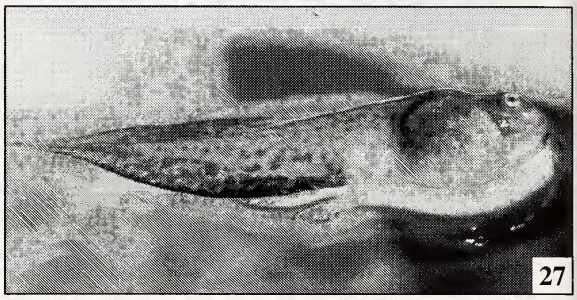

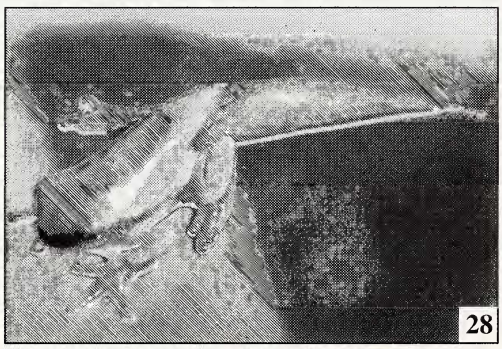

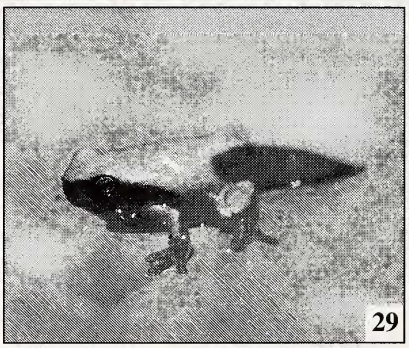

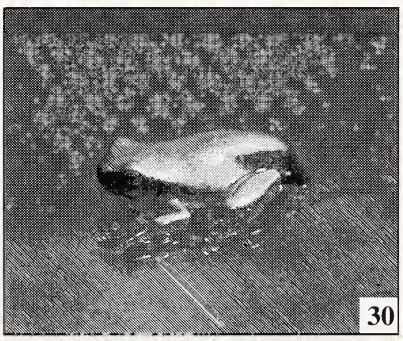

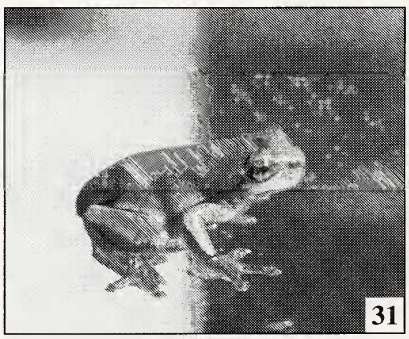

Fig. 26, 27 Cloacal tail piece reduced, Fig. 28 Both fore limbs emerged,
Fig. 29 Angle of mouth reached the middle of eye,
Fig. 30 Angle of mouth reached posterior margin of the eye, Fig. 31 Metamorphosed froglet.

Fig. 24). The margin of the 5th toe web is directed towards the tip of the 2nd toe.

Stage 37: *Margin of 5th toe web directed towards the tip of 1st toe* (Age 1084 hrs; Head and Trunk 13.7 mm; Tail 28.0 mm). The margin of the 5th toe web is directed towards the tip of the 1st toe. Pigmentation appears in the 4th and 5th toes along the foot. Toes are longer. A rudiment of prehallux is indicated by a light protuberance behind the 1st toe. Ventral surface of the foot is closer to the ventral fin.

Stage 38: *Margin of 5th toe web directed towards prehallux* (Age 1136 hrs; Head and Trunk 14.0 mm; Tail 29.00 mm). (Plate 3, Fig. 25). The margin of the 5th toe web is directed towards prehallux. Inner metatarsal turbercle appear as a small outgrowth. Pigmentation appears in 3rd, 4th and 5th toe along the foot.

Stage 39: *Appearance of subarticular tubercles in the toes* (Age 1210 hrs; Head and Trunk 14.3 mm; Tail 29.2 mm). Subarticular tubercles appear on the inner surface of the toes as light patches. The inner metatarsal tubercle becomes a small oval outgrowth.

Stage 40: *Toe pads complete* (Age 1274 hrs; Head and Trunk 14.4 mm; Tail 29.2 mm). Distal ends of the toes thickened. Subarticular tubercles are clearly elevated. The cloacal tail piece is not reduced.

Stage 41: *Cloacal tail piece reduced* (Age 1392 hrs; Head and Trunk 14.8 mm; Tail 30.0 mm. (Plate 4, Fig. 26, 27). The cloacal tail piece gets reduced and only a narrow strip remains over and in between bases of the thigh still attached with ventral fin distally. The fore limbs are visible through the skin. Green pigmentation of the dorsal surface begins. Tail not dark. Keratodonts start shedding. Oral papillae remain intact.

Stage 42: *Both fore limbs emerge* (Age 1422 hrs; Head and Trunk 14.5 mm; Tail 25.1 mm) (Plate 4, Fig. 28). Both fore limbs emerge, usually the right fore limb emerges first, followed after a few hours by the left. Resorbtion of the labial fringe begins, however angular papillae still remain as a small tuft on both corners of the mouth, which starts widening. The horny beak is shed. Tail starts darkening. The angle of the mouth is level with the nostril. The cloacal tail piece disappears at this stage, leaving the cloacal aperture free below.

Stage 43: *Angle of mouth between the eye and nostril* (Age 1434 hrs; Head and Trunk 14 mm; Tail 16.0 mm). The widening angle of mouth has reached a point midway between nostril and the anterior margin of eye. The tail becomes still darker, the dorsal and ventral fins shrink and the length of the tail is reduced, but still longer than the extended hind limb.

Stage 44: *Angle of mouth reached the middle of eye* (Age 1462 hrs; Head and Trunk 14.00 mm; Tail 5.0 mm (Plate 4, Fig. 29). The widening angle of the mouth has reached the level of the middle of the eye. Dorsal and ventral fins have disappeared. Tail resorbed considerably and is as long as the femur.

Stage 45: *Angle of the mouth reached posterior margin of the eye* (Age 1498 hrs; Head and Trunk 13.5 mm; Tail 1.0 mm) (Plate 4, Fig. 30). The widening angle of the mouth has reached the posterior margin of the eye. The tail is resorbed to a small triangular stub.

Stage 46: *Metamorphosed froglet* (Age 1550 hrs; Head and Trunk 13.5 mm; Tail 0.0 mm.) (Plate 4, Fig. 31). The triangular tail stub with dark tissue disappears completely.

Total time taken for completion of metamorphosis was 64 days 14 hours.

Discussion

Hamburger (1947), Gosner (1960), Rugh (1962), Nieuwkoop and Faber (1967) were referred for preparing the normal table. Gosner (1960) proposed 46 stages, with simplified criteria for staging developmental events in Pelobatids, Bufonids, Ranids and Hylids. In the

present study, the development of *Hyla annectans was* divided into these 46 stages. Certain variations have been noted in the development of *Hyla annectans.*

Developmental, stages have been divided into fifteen major subheadings (1) Fertilization two stages (2) Cleavage seven stages (3) Gastrula three stages (4) Neurula four stages (5) Tail bud one stage (6) Muscular response one stage (7) Heart beat one stage (8) Gill circulation one stage (9) Cornea becoming transparent one stage (10) Tail fin circulation one stage (11) Operculum formation three stages (12) Hind limb bud five stages (13) Identation and development of toes ten stages (14) Cloacal tail piece reduced one stage (15) Metamorphosis climax five stages.

Characteristic features of development include the tail bud, initially indicated by a strong upward arching of the dorsum. Hatching of the embryos occur in stage 21, when the cornea just begins to be transparent. However, the cornea becomes fully clear only towards the end of stage 22 and beginning of stage 23. At stage 22, the circulation in the tail fin begins, but the tail fin is not transparent as in Gosner series and remains dusky. Narrow cloacal tail piece persists, in Gosner series - 41 which, however, disappears only in stage 42 when both forelimbs have emerged. Rostrodont and keratodonts are shed completely at stage 42.

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